Biochimica et Biophysica Acta 896 (1987) 26–34
Elsevier

BBA 73200

Membrane lipid changes in erythrocytes, liver and kidney in acute and chronic experimental liver disease in rats

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(Received 11 September 1986)

Key words: Membrane lipid; Liver disease; Cholesterol; Arachidonate; Brush-border membrane; (Rat)

Lipid molecules in lipoprotein surfaces exchange with their counterparts in cell plasma membranes. In human or experimental liver disease, plasma lipoprotein surfaces are enriched in cholesterol and deficient in arachidonate; corresponding alterations occur in membrane lipids of erythrocytes. To determine whether similar changes take place in membranes of nucleated cells, the lipid content of plasma and of erythrocyte, liver and kidney membranes was measured in rats with acute (3-day) galactosamine-induced hepatitis or chronic (3-week) biliary obstruction. In both models of liver injury the cholesterol:phospholipid ratio in plasma and in erythrocytes was significantly increased ($P < 0.001$). Although this ratio was also elevated in liver and kidney microsomes, only in liver microsomes of obstructed rats was the increase significant ($P < 0.001$). However, the cholesterol:phospholipid ratio of kidney brush-border membranes, was significantly higher in bile-duct-ligated rats; presumably, compensating mechanisms limit cholesterol accumulation in intracellular membranes. Kidney brush-border membranes from obstructed rats were deficient in arachidonate as were plasma and erythrocytes. However, arachidonate levels were unchanged in kidney microsomes; renal Δ^6-desaturase, the rate-limiting enzyme in the conversion of linoleic acid to arachidonic acid, was increased by 50% ($P < 0.001$) and may have counteracted a reduced supply of exogenous lipoprotein arachidonate. We conclude that in experimental liver disease lipoprotein-induced lipid abnormalities can occur in renal membranes, although compensatory mechanisms may operate; the alterations seen, cholesterol accumulation and arachidonate depletion, would be expected to interfere with sodium transport and prostaglandin production, respectively. Our findings support the hypothesis that lipid abnormalities in kidney membranes contribute to the renal dysfunction which is a frequent complication of human liver disease.

Introduction

In both human and experimental liver disease, characteristic changes occur in the lipid composition of the surface coat of plasma lipoproteins. These include a reduction of arachidonyl fatty acyl chains in the phospholipids and, as a consequence of diminished hepatic synthesis and/or secretion of lecithin–cholesterol acyltransferase (EC 2.3.1.43), an accumulation of cholesterol and phosphatidylcholine molecules [1]. Cholesterol and phospholipid molecules in lipoprotein surfaces tend to exchange and equilibrate with their counterparts in cell plasma membranes [2,3] and it is not surprising, therefore, that in liver disease,

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platelets [4,5] and erythrocyte membranes [6–9] show lipid abnormalities similar to those of the plasma lipoproteins.

The erythrocyte and platelet lipid abnormalities seen in jaundiced patients appear to correlate with changes in cellular and cell membrane functions. Cholesterol enrichment of the erythrocyte membrane decreases its fluidity [9] and there is an associated impairment of Na^+ transport [8,10] and inhibition of anion exchange via the band 3 transmembrane protein [11]. Platelets from patients with liver disease aggregate less readily [4], apparently because there is insufficient arachidonic acid in platelet membrane phospholipids to convert to prostaglandins and thromboxane A_2 [4,12]. It seems likely, though experimentally unproven, that such membrane lipid abnormalities occur in tissues and organs and that membrane dysfunction will be widespread in liver disease.

Patients with severe liver disease develop several renal functional abnormalities, and may retain Na^+ and water [13–17]. No obvious histological changes are seen in the kidneys and the cause of the renal dysfunction is uncertain, since many of the proposed mechanisms may be secondary rather than primary events in their genesis [13–15]. One possibility is that renal membrane lipid abnormalities play an important role; accumulation of cholesterol may directly interfere with Na^+ transport, whilst reduced arachidonic acid levels may limit production of renal prostaglandins, including the vasodilator prostaglandin E_2 which helps to regulate renal vascular resistance, affects renal Na^+ and water excretion, and interacts with the renin-angiotensin system [16,17]. As a preliminary step in testing the hypothesis that membrane lipid changes contribute to renal dysfunction in liver disease, we have measured the cholesterol content and phospholipid fatty acid composition in kidney microsomes and brush-border membranes in rats with either acute galactosamine-induced hepatitis or chronic biliary obstruction.

Materials and Methods

Experimental animals

Male Sprague-Dawley rats initially weighing 180–200 g were used; they were fed on commercial rat chow. Two models of liver injury were employed.

(i) Galactosamine hepatitis. Rats were given intraperitoneal D-galactosamine hydrochloride (1 g/kg body weight) dissolved in saline on the 1st and 3rd days. Control rats were given saline alone and all were killed on the 4th day.

(ii) Bile duct ligation. Double ligation of the common bile duct, with section between the two ligatures, was performed under diethyl ether anaesthesia. Other rats were subjected to sham-operations and both groups were studied 3 weeks after the operations.

Preparation of microsomes from liver and kidney and of brush-border membranes from kidney cortex

Rats were killed by diethyl ether inhalation and their liver and kidneys were perfused in situ with 100 ml of ice-cold 10 mM Tris-HCl buffer (pH 7.5)/0.25 M sucrose. Microsomes were obtained from a cell-free homogenate by centrifugation at $105\,000 \times g$ for 1 h at 4°C as described previously [18]. The microsomal pellet obtained was suspended in buffer and washed once by re-centrifugation. One portion was used immediated for lipid analysis, while another was stored at −75°C for assay of Δ^6-desaturase activity.

For preparation of the brush-border membrane the kidneys were placed on ice and the medulla removed by dissection. The remaining cortex was homogenized in 50 mM mannitol and, following addition of 0.4 ml of 1 M $CaCl_2$, the final volume was adjusted to 40 ml. The suspension was gently mixed for 20 min and centrifuged at $3000 \times g$ for 10 min at 4°C. The supernatant was removed and recentrifuged at $18\,000 \times g$ for 10 min to pellet the brush-border membrane [19]. Protein concentrations were determined with the Folin-Ciocalteau reagent [20] using bovine serum albumin as standard.

Lipid analyses

Lipids were extracted from plasma by the procedure of Folch et al. [21], from washed erythrocytes with isopropanol and chloroform [22] and from microsomes and brush-border membranes by the method of Bligh and Dyer [23]. Plasma free and total cholesterol concentrations were measured by gas-liquid chromatography as described

previously [24] and membrane cholesterol and plasma triacylglycerols using commercial enzymatic kits (Boehringer Corporation Ltd., Lewes, East Sussex, U.K.). Lipid phosphorus was measured as inorganic phosphorus following digestion with H_2SO_4 at 180°C [25]. The phospholipid fatty acid composition of microsomes and brush-border membranes was determined by gas-liquid chromatography following separation of total phospholipids from neutral lipids on silica-gel G (Merck) thin-layer chromatograms with hexane/diethyl ether/acetic acid, 90:20:1 (v/v) as solvent. The phospholipid fraction was transmethylated by heating at 90°C for 1 h under N_2 in 14% (w/v) BF_3 in MeOH. The fatty acid methyl esters were extracted into hexane and separated at 185°C on a 150 cm column of 10% EGSS-X on Gas-Chrom P, 100/120 mesh; detection was by flame ionization.

Enzyme assays

Plasma lecithin–cholesterol acyltransferase activity was assayed using endogenous lipoproteins pre-labelled with [^{14}C]cholesterol as substrate [26]. The incubation time (30 min) was within the linear period and, following separation of cholesterol and cholesteryl ester by thin-layer chromatography as described previously [27], the percentage of cholesterol esterified per h was determined.

In man, arachidonic acid (20:4($n-6$) is derived almost entirely from dietary linoleic acid (18:2($n-6$) by desaturation and chain elongation [28]. The rate-limiting step is the initial conversion of linoleic acid to γ-linolenic acid (18:3($n-6$) by a Δ^6-desaturase. This enzyme was assayed in liver and kidney microsomes by incubating microsomal protein (0.2–1.0 mg) with 50 nmol NADH, 50 μmol potassium phosphate buffer (pH 7.4) and 50 nmol [^{14}C]linoleoyl-CoA (0.1 μCi) in a final volume of 0.6 ml for either 10 min (liver) or 60 min (kidney) at 37°C. Reactions were stopped by addition of $CHCl_3$/MeOH (1:2, v/v) [22]. The lipid extracts were dried and the fatty acids transmethylated for 1 h at 90°C with 14% (w/v) BF_3 in MeOH. The methyl esters were extracted into hexane and separated on silica-gel H thin-layer chromatograms containing 10% (w/w) $AgNO_3$ [29]. The esters of linoleic acid and linolenic acid were removed and the percentage conversion was calculated from the distribution of ^{14}C between substrate and product fatty acids.

Statistics

All results are expressed as means ± S.E.; statistical differences were determined by the unpaired Student's *t*-test.

Results

Plasma lipids and lecithin–cholesterol acyltransferase activity

The plasma concentrations of triacylglycerol were significantly reduced ($P < 0.001$) in both models of liver injury, but total phospholipid levels were unchanged (Table I). Total cholesterol (i.e., free cholesterol and cholesteryl esters) was 25% higher ($P < 0.05$) in bile-duct-ligated rats than in sham-operated animals, but was unchanged in galactosamine-treated rats when compared to their controls. Plasma lecithin–cholesterol acyltransferase activity was significantly reduced in both galactosamine-treated and bile-duct-ligated rats, whether expressed as an absolute or fractional amount of cholesterol converted into cholesteryl esters. As expected, in both galactosamine-treated and bile-duct-ligated rats, these reduced lecithin–cholesterol acyltransferase activities resulted in significant increases ($P < 0.001$) in the proportion of plasma total cholesterol present as free cholesterol and in the cholesterol:phospholipid molar ratio, *C/PL*. The proportions of linoleate, dihomo-γ-linoleate (20:3($n-6$) and arachidonate in the plasma total fatty acyl chains of bile-duct-ligated rats were significantly decreased ($P < 0.05$) compared to those in the sham-operated rats (20.6 ± 0.8, 0.7 ± 0.1 and 9.6 ± 0.5% compared to 27.2 ± 0.5, 1.3 ± 0.1 and 11.0 ± 0.3%, respectively). In the galactosamine-treated rats these proportions were unchanged compared to the control rats.

Lipid content of erythrocytes and microsomal membranes of liver and kidney

The cholesterol content per mg protein was significantly higher ($P < 0.001$) in liver microsomal membranes in both galactosamine-treated and bile-duct-ligated rats (Table II). However, in kidney microsomes the increase in mean

TABLE I

PLASMA LIPID COMPOSITION AND LECITHIN-CHOLESTEROL ACYLTRANSFERASE (LCAT) ACTIVITY IN RATS WITH ACUTE GALACTOSAMINE-INDUCED HEPATITIS OR CHRONIC BILIARY OBSTRUCTION

Groups of rats were injected with D-galactosamine hydrochloride (GalN) or saline and killed 3 days later or were subjected to bile-duct ligation (BDL) or sham-operation for 3 weeks as described in Materials and Methods. Results are means ± S.E. and significance of differences from control values are indicated in the footnotes.

Animals (No.)	Triacylglycerol (mmol/l)	Total phospholipid (mmol/l)	Total cholesterol (mmol/l)	Percentage of free cholesterol (%)	LCAT activity		Cholesterol/ phospholipid ratio (mol/mol)
					%/h	nmol/ml per h	
GalN (13)	0.64 ± 0.08 ***	1.55 ± 0.09	1.50 ± 0.09	61.4 ± 3.4 ***	4.9 ± 0.7 ***	41.6 ± 5.7 *	0.61 ± 0.05 **
Saline-injected (6)	1.91 ± 0.21	1.63 ± 0.06	1.47 ± 0.12	31.0 ± 0.7	14.1 ± 1.5	62.9 ± 6.2	0.28 ± 0.02
BDL (7)	0.72 ± 0.04 ***	1.41 ± 0.11	1.71 ± 0.13 *	43.2 ± 1.7 ***	4.9 ± 0.1 ***	36.0 ± 2.7 ***	0.52 ± 0.01 ***
Sham-operated (8)	1.93 ± 0.33	1.64 ± 0.03	1.37 ± 0.05	29.0 ± 0.3	17.2 ± 0.8	67.2 ± 2.2	0.24 ± 0.01

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

cholesterol content was less than 10% and reached significance ($P < 0.05$) only in the bile-duct-ligated rats. Phospholipid content per mg of liver or kidney microsomal protein was unchanged, except in liver microsomes of galactosamine-treated rats which showed a small increase ($P < 0.05$).

Membrane lipid analyses are better expressed as ratios or proportions, rather than absolute amounts per cell or per mg of membrane protein, since the former are good indicators of lipid–lipid interactions within biomembranes. For example, in erythrocytes from patients with liver disease reduced membrane fluidity correlates closely with the *C/PL* molar ratio but not with the cholesterol content per mg of membrane protein [9]. In both bile-duct-ligated and galactosamine-treated rats the *C/PL* ratios of erythrocyte membranes were significantly elevated ($P < 0.001$) (Table II). The mean *C/PL* ratios of kidney and liver microsomal membranes were higher in bile-duct-ligated and galactosamine-treated rats than in the corresponding controls, suggesting that their fluidity may have been reduced. However, the rises in the *C/PL* ratio were small and not significant ($0.05 < P < 0.01$), except for liver microsomes of bile-duct-ligated rates, which showed a marked increase ($P < 0.001$).

Phospholipid fatty acyl chains in erythrocytes and in microsomal fractions of liver and kidney

The proportion of arachidonate in erythrocyte phospholipid fatty acyl chains was significantly lower in the galactosamine-treated or bile-duct-ligated rats, albeit less than 10%, than in the corresponding controls (Table III). However, this reduction was not reflected in the phospholipids of liver or kidney microsomes, since no decrease in arachidonate was seen in either experimental

TABLE II

LIPID COMPOSITION OF LIVER AND KIDNEY MICROSOMES AND OF ERYTHROCYTE MEMBRANES FROM RATS WITH ACUTE GALACTOSAMINE-INDUCED HEPATITIS OR CHRONIC BILIARY OBSTRUCTION

Groups of rats and presentation of results are as described in Table I. Values in nmol/mg protein. *C/PL*, cholesterol:phospholipid molar ratio.

Animals (No.)	Liver microsomes			Kidney microsomes			Erythrocyte membranes (*C/PL*)
	cholesterol	phospholipid	*C/PL*	cholesterol	phospholipid	*C/PL*	
GalN (13)	128 ± 5	822 ± 40	0.15 ± 0.01	210 ± 2	586 ± 6	0.36 ± 0.00	0.84 ± 0.01 ***
Saline-injected (6)	97 ± 2	702 ± 26	0.14 ± 0.00	194 ± 14	562 ± 32	0.34 ± 0.01	0.73 ± 0.00
BDL (7)	169 ± 8 ***	668 ± 19	0.25 ± 0.01 ***	222 ± 2 *	570 ± 20	0.39 ± 0.02	0.82 ± 0.01 ***
Sham-operated (8)	96 ± 4	720 ± 15	0.13 ± 0.01	207 ± 5	549 ± 13	0.38 ± 0.00	0.74 ± 0.01

* $P < 0.05$. *** $P < 0.001$.

TABLE III

COMPOSITION OF PHOSPHOLIPID FATTY ACYL CHAINS IN ERYTHROCYTE MEMBRANES AND KIDNEY AND LIVER MICROSOMES FROM RATS WITH ACUTE GALACTOSAMINE-INDUCED HEPATITIS OR CHRONIC BILIARY OBSTRUCTION

Groups of rats are as described in Table I. Results (weight %) of major fatty acids with retention times up to and including arachidonic acid (20:4($n-6$) are means ± S.E. and significance of differences from control values are indicated in the footnotes.

Animals	(No.)	Erythrocyte membranes					
		16:0	18:0	18:1	18:2	20:3	20:4
GalN	(13)	33.9 ±0.2	11.6 *** ±0.1	12.8 ±0.2	13.4 ** ±0.2	1.9 * ±0.1	26.3 * ±0.2
Saline-injected	(6)	32.7 ±0.9	12.6 ±0.2	12.9 ±0.5	11.1 ±0.5	2.4 ±0.1	28.5 ±0.7
BDL	(7)	31.6 ±0.1	10.5 *** ±0.5	17.2 *** ±0.5	8.9 ** ±0.4	1.7 ±0.5	26.2 * ±0.4
Sham-operated	(8)	32.7 ±0.4	13.7 ±0.3	13.0 ±0.3	10.9 ±0.4	2.0 ±0.5	27.7 ±0.4

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

Liver microsomes						Kidney microsomes					
16:0	18:0	18:1	18:2	20:3	20:4	16:0	18:0	18:1	18:2	20:3	20:4
22.7 *** ±0.4	25.3 ±0.6	9.6 ±0.2	18.1 ±0.3	1.9 ** ±0.2	22.7 ±0.5	21.7 ** ±0.9	19.0 ±0.2	11.7 ±0.1	15.6 ±0.4	2.6 ±0.4	29.6 * ±0.9
24.9 ±0.2	24.1 ±0.5	9.1 ±0.2	16.7 ±0.6	3.0 ±0.2	22.3 ±0.4	24.5 ±0.2	18.2 ±0.4	11.1 ±0.3	16.1 ±0.4	2.8 ±0.4	27.5 ±0.5
25.8 * ±0.8	19.9 *** ±0.2	18.7 *** ±0.4	11.7 ** ±0.5	1.5 *** ±0.2	21.6 ±1.1	24.4 ±0.6	19.8 ±0.9	14.3 *** ±0.6	11.6 *** ±0.6	2.4 ±0.3	27.5 ±1.2
22.6 ±0.6	25.5 ±0.5	9.7 ±0.3	16.8 ±0.5	2.9 ±0.2	22.0 ±0.4	24.6 ±0.6	19.0 ±0.2	11.0 ±0.1	15.5 ±0.3	1.9 ±0.1	28.0 ±0.8

model. Linoleate, the main dietary precursor of arachidonate, was significantly reduced in the phospholipids of both liver and kidney microsomes in bile-duct-ligated rats but it was unchanged in galactosamine-treated animals.

Microsomal Δ^6-desaturase activity in liver and kidney

The Δ^6-desaturase activities in microsomal fractions from normal rat liver (107–270 pmol linoleic acid converted per min per mg protein) were similar to those reported by other workers [30,31] and at least 4-fold greater than in fractions from kidney (Table IV). The mean activity in liver microsomes was significantly lower in both galactosamine-treated rats ($P < 0.001$) and bile-duct-ligated rats ($P < 0.05$) when compared to their control groups. Unexpectedly, the mean value in

TABLE IV

MICROSOMAL Δ^6-DESATURASE ACTIVITY IN LIVER AND KIDNEY FROM RATS WITH ACUTE GALACTOSAMINE-INDUCED HEPATITIS OR CHRONIC BILIARY OBSTRUCTION

Groups of rats and presentation of results are as described in Table I.

Animals (No.)	Microsomal Δ^6-desaturase activity (pmol/min per mg protein)	
	liver	kidney
GalN (13)	131 ± 12 ***	19.1 ± 1.6
Saline-injected (6)	270 ± 35	22.0 ± 1.8
BDL (7)	106 ± 4 *	23.3 ± 1.8 **
Sham-operated (8)	132 ± 7	15.2 ± 1.0

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

TABLE V

LIPID COMPOSITION IN KIDNEY BRUSH-BORDER MEMBRANE FROM RATS WITH CHRONIC BILIARY OBSTRUCTION

Groups of rats were subjected to either bile-duct ligation (BDL) or sham-operation and killed 3 weeks later. Results are means ± S.E. for the numbers in parentheses and significance of differences between the groups are indicated in the footnotes. *C/PL*, cholesterol:phospholipid molar ratio.

Animals	(No.)	Cholesterol (nmol/mg protein)	Phospholipid (nmol/mg protein)	*C/PL*	Phospholipid fatty-acid composition (wt%)					
					16:0	18:0	18:1	18:2	20:3	20:4
BDL	(7)	429 ± 9 ***	643 ± 15 **	0.668 ± 0.009 *	24.7 ± 0.8	23.9 ± 0.8 **	10.2 ± 0.6	8.5 ± 0.5	2.5 ± 0.7	28.9 ± 0.6 ***
Sham-operated	(6)	343 ± 9	545 ± 21	0.631 ± 0.013	25.9 ± 0.4	22.7 ± 0.6	7.4 ± 0.4	8.6 ± 0.5	2.0 ± 0.5	33.4 ± 0.4

* $P < 0.05$. ** $P < 0.01$. *** $P < 0.001$.

the sham-operated rats was half that in the group injected with saline as control for the galactosamine-treated rats, even though all samples were assayed in the same run. The reason for this difference was not investigated. One possibility is that experiments with sham-operated (and bile-duct-ligated) rats lasted 18 days longer than those with saline-injected (and galactosamine-treated) rats and were begun 2 months later in the year; Δ^6-desaturase activity is known to vary with the age of the animal and with the season of the year [30], whilst the 2-month extra storage time of microsomes from saline-injected rats may also have affected enzymatic activity. In kidney microsomes the Δ^6-desaturase activity was significantly increased in bile-duct-ligated rats ($P < 0.001$) but was unchanged in the galactosamine-treated animals (Table IV).

Lipid composition of brush-border membranes in the kidney cortex of biliary-obstructed rats

To assess whether lipid changes might be more pronounced in kidney plasma membranes, we isolated the brush-border membranes from seven bile-duct-ligated rats. The cholesterol content and *C/PL* ratio were both significantly raised in these membranes (Table V), although we had been unable to detect a change in the *C/PL* ratio of the microsomal fraction (Table II). In addition, the amount of arachidonate in brush-border membrane phospholipids was 15% lower in bile-duct-ligated rats compared to sham-operated controls ($P < 0.001$), but there was no difference in the microsomal fractions.

Discussion

Results from the present study establish for the first time that lipid abnormalities occur in renal membranes in experimental liver disease; they appear to be induced by changes in plasma lipoprotein lipid composition. Rat kidney brush-border membranes were found to have accumulated cholesterol and to have lost arachidonate in phospholipid fatty acyl chains 3 weeks after bile-duct ligation, alterations expected to interfere with Na^+ transport and to diminish prostaglandin synthesis, respectively. These findings provide support for the hypothesis that lipid abnormalities in kidney membranes contribute to the renal dysfunction which is commonly seen in patients with severe liver disease.

As expected from previous studies [32–34], liver injury in rats induced by either bile-duct ligation or galactosamine treatment resulted in a fall in plasma lecithin–cholesterol acyltransferase activity, an accumulation of cholesterol in lipoprotein surfaces and a rise (of up to 15%) in the cholesterol : phospholipid ratio of red cell membranes. Liver microsomes from bile-duct-ligated rats were also enriched in cholesterol, but no change was found in those from galactosamine-treated rats. This difference was unexpected; cholesterol exchange between lipoproteins and membranes is rapid [2] and the red cell membranes of both groups were enriched to a similar extent. Nucleated cells have two ways to handle a net influx of exogenous cholesterol which are unavailable to red cells; they can reduce endogenous cholesterol synthesis and they can divert cholesterol from their membrnes into an intracellular cholesteryl ester storage pool. It is conceivable that these mechanisms were still able to function during the 3-day period of galactosamine treatment but were impaired by prolonged biliary obstruction.

Kidney microsomes in bile-duct-ligated rats, however, accumulated no more cholesterol than those in galactosamine-treated animals; in both cases the mean rise in the *C/PL* ratio was small (less than 4%) and statistically insignificant. Kidney cells differ from those in liver by being exposed only to the lipoproteins of the extravascular fluid; fenestrations in the thin layer of sinusoidal endothelium enables all of the plasma lipoproteins, except chylomicrons and large very-low density lipoproteins, to interact with the hepatocyte surface [35]. The relationship between lipoproteins in plasma and in interstitial fluid is poorly defined; current evidence suggests that as well as differences in concentration there may be dissimilarities in composition and morphology of particles of the same lipoprotein class [36,37]. Cholesterol uptake by red-cell membranes from cholesterol-rich plasma in vitro depends on the incubation time, the *C/PL* ratio of the plasma and the relative amounts of membrane cholesterol and plasma cholesterol [38]. Whether the inability of kidney microsomes in bile-duct-ligated rats to amass

cholesterol compared with liver microsomes can be attributed to diminished concentrations and/or lower *C/PL* ratios of renal interstitial fluid lipoproteins compared to plasma lipoproteins remains to be established. The plasma membrane forms only a small percentage of a microsomal fraction and, although cholesterol equilibrates relatively freely between surface membranes and membranes of intracellular organelles [2], it is possible that our analysis of whole kidney microsomes may have masked a greater cholesterol enrichment of the plasma membrane. Analysis of brush-border membranes from the kidney cortex of bile-duct-ligated rats supported this possibility; their *C/PL* ratio was significantly raised, indicating that brush-border membranes cannot maintain a normal cholesterol content when kidney cells are exposed to abnormal, cholesterol-rich lipoproteins.

Most arachidonic acid in mammalian cells is present as an ester in the 2-position of glycerophospholipids. It is derived predominantly from dietary linoleic acid by desaturation and chain elongation; the initial introduction of a double bond by Δ^6-desaturase, to form γ-linolenic acid, is the rate-limiting step [28,39,40]. In rat, and probably man, liver has the highest desaturase activity [40]. It has been suggested that the liver could be the major source of arachidonic acid for other tissues in the rat [40]; this function probably involves lipoproteins as transport vehicles, since circulating complexes of albumin and free-fatty acids contain relatively little arachidonic acid [41].

Red cells cannot synthesize fatty acids or elongate or desaturate fatty acyl chains, turnover of these lipids occurs by utilization of exogenous free-fatty acids in the phospholipid deacylation-reacylation cycle and by equilibrium exchange of intact phospholipid molecules between membrane and plasma. It is not surprising, therefore, that there is a significant reduction of arachidonate in the phospholipids of red cell membranes in bile-duct-ligated rats and that this reflects the decreased proportion of arachidonate in plasma. The reasons for plasma arachidonate deficiency are less clear [42]. Abnormal lipoprotein metabolism may be one factor, inasmuch as patients with familial lecithin–cholesterol acyltransferase deficiency have good liver function but reduced arachidonate levels in plasma, red cells and platelets [43]. Impaired absorption of dietary linoleic acid may also contribute, but decreased hepatic conversion of linoleate to arachidonate probably plays the predominant role. The reduced Δ^6-desaturase activity in liver of bile-duct-ligated rats suggests reduced production of arachidonic acid and decreased availability for export in the phospholipids of lipoprotein surfaces. Despite this diminished Δ^6-desaturase activity, normal arachidonate levels were found in the phospholipids of hepatic microsomes; the liver may be able to conserve this eicosanoid precursor [44]. The proportion of arachidonate in phospholipids of kidney microsomes was also unchanged in bile-duct-ligated rats. However, when renal brush-border membranes were analysed a significant reduction in arachidonate was found; conceivably, the 50% increase in Δ^6-desaturase activity in kidney microsomes of bile-duct-ligated rats could represent a mechanism to compensate for a diminished exogenous supply of lipoprotein arachidonate.

In conclusion, our analyses of renal brush-border membranes establish that both the cholesterol and fatty-acid contents of these membranes can be influenced by the lipid composition of interstitial fluid lipoproteins. Similar alterations in the lipids of intracellular renal membranes were not detected, suggesting that compensatory mechanisms, such as changes in lipid synthetic rates, may have been operating. Whether these putative mechanisms can adequately compensate if cellular exposure to the altered lipoproteins is more prolonged is uncertain; gross cholesterol accumulation occurs in the renal cortex of patients with familial lecithin–cholesterol acyltransferase deficiency [43], but in mice given fat-free diets the renal cortex tenaciously retains arachidonate in all phospholipid classes except phosphatidylinositol [44]. By analogy with studies in red cells [8,10] and platelets [4,12] from jaundiced patients, cholesterol enrichment and arachidonate depletion of renal membranes are likely to perturb Na^+ transport and prostaglandin production, respectively; whether kidney membrane lipid abnormalities play a significant role in the impaired renal handling of sodium and water reported in biliary-obstructed rats [45] or can contribute to a better understanding of the aetiology of renal dysfunction in human liver disease must await additional investigations.

Acknowledgement

This work was supported by a grant from the Medical Research Council.

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